Gaseous Myoglobin Ions Stored at Greater than 300 K

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Multiply-charged myoglobin ions retaining the prosthetic heme group have been formed by electrospray, injected into a quadrupole ion trap, and stored for up to one second prior to mass analysis. Collisional activation experiments indicate that these ions readily fragment into the charged heme group and the complementary apomyoglobin ion. No fragmentation is observed, however, upon ion storage in the presence of a neutral bath gas at 1×10^{-3} torr for up to one second. The significance of this observation is that these non-covalently-bound ions, in which both the heme group and the polypeptide carry charge, are kinetically stable for over one second at room temperature and, perhaps, at higher temperatures. This suggests that other biologically relevant ions derived using electrospray and bound by non-covalent interactions can be studied using the various tools available with ion storage mass spectrometers and by other techniques that employ relatively high pressure environments for the study of gaseous ions. *(J Am Soc Mass Spectrom 1994, 5, 324–327)*

Gaseous ions comprised of biomolecular species bound by non-covalent interactions have recently been reported using electrospray [1–15]. The ability to form such species is particularly significant due to the importance of non-covalent interactions in biological systems. Examples include peptide-protein complexes [1,2,5], DNA duplexes [8,9] and quadruplexes [7], and protein-prosthetic group complexes [3,12,13,15]. The significance of their existence in the gaseous state derives largely from the fact that they can be studied via mass spectrometry, a powerful tool for molecular weight and structural information. Characteristically, biopolymeric ions derived from electrospray are multiply-charged [16–20] and can therefore be expected to be destabilized by internal coulombic repulsion [21] when the individual components of the complex each carry a net charge. To date, virtually all non-covalently bound multiply-charged biomolecule clusters have been observed using beam-type mass spectrometers, principally quadrupole mass filters, wherein ion lifetimes of up to several hundred microseconds are required for observation of the intact ions. Recently, several examples in which non-covalently bound biomolecule complexes have been stored in the low pressure environment of the ion cyclotron resonance spectrometer have been reported [22]. This report describes the trapping and storage of multiply-charged myoglobin for periods up to at least one second in the presence of a relatively high pressure bath gas in a quadrupole ion trap. For these ions to be observed retaining the non-covalently bound heme group they must survive passage through the atmosphere/vacuum interface, as they also must with beam-type mass spectrometers, and must also survive the processes of ion trapping, ion storage, and mass analysis. Each of the latter events provides opportunity for ion dissociation, particularly in the quadrupole ion trap operated in the presence of a bath gas at roughly one millitorr.

Experimental

A 6 μM solution of myoglobin (Aldrich, St. Louis, MO) in water was infused at a rate of 2 μL/min through a 120 μm-i.d. (500 μm o.d.) dome-tip needle within a home-made pneumatically-assisted electrospray source. All experiments were carried out using an Ion Trap Mass Spectrometer (Finnigan, San Jose, CA) modified for electrospray. Details of the apparatus and procedures for acquiring a mass spectrum have been described [23]. Spectra reported here are the average of 360 individual scans with each scan, including ion accumulation, requiring roughly one second. The mass/charge range of the Ion Trap Mass Spectrometer was extended by a factor of five via resonance ejection. Given the fixed scan rate of 5555 Da/s of this system, extending the mass/charge range also increases the scan speed in equal proportion. Mass spectrometry/mass spectrometry results were obtained using two resonance ejection periods to select the mass-to-charge region of interest followed by conventional ion trap resonance excitation to effect collisional activation [24].

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A wide mass/charge range was isolated to avoid collisionally exciting the parent ions in the mass-selection steps [25].

Results and Discussion

Ionized myoglobin is one of the first and most widely studied weakly-bound species formed via electrospray [3,12,13,15]. Katta et al. showed that the native form of the protein could be ionized under neutral pH conditions [3]. Much larger signals and more highly charged ions are observed for apomyoglobin, however, using solutions prepared at low pH [3,12,13,15]. Electrospray mass spectra of apomyoglobin have already been reported using ion trapping instruments [23, 26–32]. Figure 1 shows the electrospray mass spectrum of a 6 μM solution of myoglobin in water employing an ion accumulation period of 0.8 s prior to mass analysis. The spectrum shows prominent signals due to the +9, +8, and +7 charge states of myoglobin. Smaller signals are observed for apomyoglobin at +8 and +7 charge states as well as the singly-charged heme group at m/z 616. The relative intensities of the signals due to the heme group and the apomyoglobin ions are highly dependent upon the atmosphere/vacuum interface conditions of pressure and electrode potentials. This suggests that these ions arise from fragmentation in the interface as has been noted previously with a beam-type spectrometer [12]. However, dissociation might also occur upon ion injection into the ion trap, during ion storage, or in the process of mass analysis. For example, fragmentation has been observed for some covalently-bound ions injected into the ion trap from external ion sources [33–35]. This fragmentation is attributed to internal excitation of the injected ions via inelastic collisions with the bath gas as they are kinetically cooled to the center of the ion trap. Small weakly bound ions, such as the molecular ion of tetraethylsilane [36] and protonated water clusters containing three or more water molecules [37], have been shown to fragment during ion storage in the ion trap. Ion storage in the presence of a bath gas brings the internal temperature of trapped polyatomic ions to at least the temperature of the bath gas and perhaps higher due to rf heating [38]. Ions can also be induced to fragment due to inelastic collisions in the ion ejection process that takes place during mass analysis [26].

Figure 2a shows the MS/MS spectrum of the +8 charge state of myoglobin acquired after mass selection of the m/z 2190–2310 region and collisional activation of the +8 parent ion. Two product ions are observed corresponding to the ionized heme group and the complementary +7 apomyoglobin ion. The product ion peaks are noticeably narrower than the peak associated with the isolated parent ions because

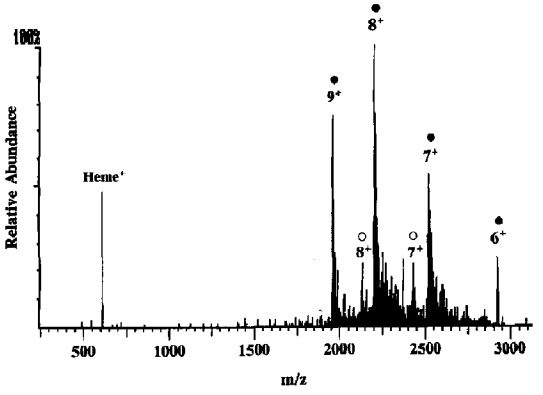

Figure 1. Electrospray mass spectrum of a 6 μM solution of myoglobin in water acquired with a quadrupole ion trap following an ion accumulation time of 0.8 s. Multiply-protonated myoglobin is represented by filled circles, whereas multiply-protonated apomyoglobin is represented by open circles.

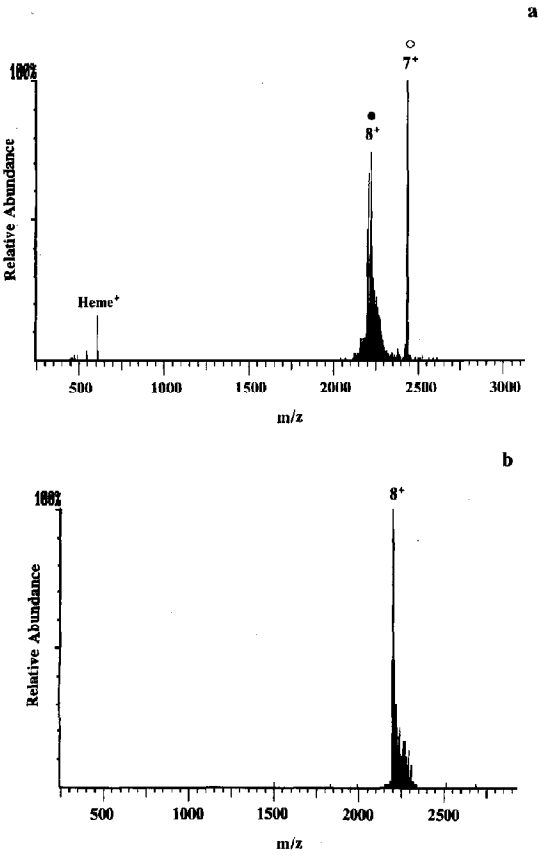

Figure 2. (a) MS/MS spectrum of the +8 charge state of myoglobin employing a 300 mV amplitude dipolar resonance excitation signal for 100 ms (q_z = 0.19). Multiply-protonated myoglobin is represented by a filled circle, whereas multiply-protonated apomyoglobin is represented by an open circle. (b) MS/MS spectrum of the +8 charge state of myoglobin in the absence of resonance excitation and with a storage time of 1 s at q_z = 0.19 prior to mass analysis.

the resonance excitation signal used to collisionally activate the parent ions excites a narrower range of mass-to-charge values than was isolated. (Note that no loss of neutral heme was observed, in contrast to MS/MS data acquired using a beam-type tandem mass spectrometer [12]. Ion trap collisional activation preferentially promotes energetically favored decompositions [39]. Relief of coulombic strain would favor the loss of charged heme from the complex over loss of neutral heme.) Figure 2b shows the spectrum derived by mass-selection of the ions in the m/z 2190–2310 region and storing them at a q_z value of 0.19 for one second prior to mass analysis via resonance ejection [40,41] at a rate of 27,775 Da/s. No detectable heme or +7 apomyoglobin ions are formed during this storage period. Assuming a collision rate constant of 5×10^{-10}–5×10^{-9} cm^3-molecule^{-1}-s^{-1} for this myoglobin ion[15], roughly 10^4–10^5 collisions occur with the bath gas during a storage period of 1 s. (A collision cross-section of 2.52×10^{-13} cm^2 was recently reported for the +8 charge state of apomyoglobin [42]. Assuming thermal kinetic energy, the collision rate constant for this collision cross section is 5×10^{-10} cm^3-molecule^{-1}-s^{1}. This value is expected to be conservative because the ion kinetic energy is likely to be higher than that of the bath gas and the ion-induced dipole interaction associated with the eight charge sites is expected to contribute to the collision frequency at low kinetic energies. The averaged dipole orientation rate constant calculated for a singly-charged ion of the mass of myoglobin with helium is also 5×10^{-10} cm^3-molecule^{-1}-s^{-1}. Since there are eight charges, the upper limit assumed here is roughly 5×10^{-9} cm^3-molecule^{-1}-s^{-1}.) Given such a large number of collisions in addition to those that occurred during the 0.8 s ion accumulation period, the ions are expected to have assumed the steady state internal energy distribution. This spectrum demonstrates that the ions are kinetically stable under these conditions and that they survive mass analysis at this scan rate. The latter conclusion can be drawn because any dissociation associated with resonance ejection of the +8 myoglobin to yield the +7 apomyoglobin ion would result in the appearance of the apomyoglobin ion in the spectrum because the ion trap is scanned according to increasing mass/charge. The heme ion formed during resonance ejection of the +8 myoglobin ion, although not subject to resonance ejection, would be ejected at a q_z value of 0.908 and appear at a mass/charge of 3080. No such signals were observed. This result shows that these non-covalently bound ions of biological significance can survive for long periods in the absence of solvent and in the presence of a thermalizing gas. These results are also significant in that they present opportunities for the study of non-covalently bound multiply-charged biomolecule complexes with ion trapping instruments operated at relatively high background pressures. The fact that these ions can be kinetically stable over such long time frames will allow the tools of quadrupole ion trap ion mass spectrometry, such as collisional activation, photodissociation, ion molecule reactions, etc., to be applied to this important new class of gaseous ions.

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